Review Form 3

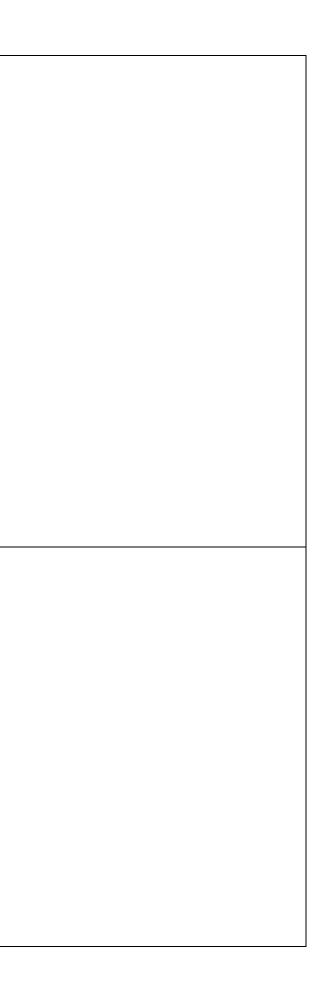|	
Journal Name:	**Journal of Cancer and Tumor International**
Manuscript Number:	**Ms_JCTI_130299**
Title of the Manuscript:	**Effect Of Rosemary Leaf Ethanol Extract (Rosmarinus Officinalis L.) On Arginase-1 And Tumor Suppressor P53 Expression Of Hepatocellular Carcinoma In Male Wistar Strain Rats (Rattus Norvegicus L.) Induced By p-Dimethylamino benzaldehyde (DMBA)**
Type of the Article	**Original Research Article**

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	Reviewer's comment	Author's Feedback *(Please correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Please write a few sentences regarding the importance of this manuscript for the scientific community. A minimum of 3-4 sentences may be required for this part.		
Is the title of the article suitable? **(If not please suggest an alternative title)**	1. **No, it's too long and complex. Its very details and multiple elements are covered for a title to be. The word effect of can be slightly changed with words like "Impact of" or simply "Effect on".** 2. **"Hepatocellular Carcinoma in Male Wistar Strain Rats", instead it can be "Hepatocellular Carcinoma in Male Wistar Strain Rats".** 3. **Full scientific names for the rat species and rosemary might not be necessary** Effect of Rosemary Leaf Extract on Arginase-1 and p53 Expression in Hepatocellular Carcinoma in Rats Or **Impact of Rosemary Leaf Extract on Arginase-1 and p53 Expression in Hepatocellular Carcinoma in Rats**	

| Is the abstract of the article comprehensive? Do you suggest the addition (or deletion) of some points in this section? Please write your suggestions here. | 1. **Aim – its comprehensive, it can be simplified with better clarity**
2. **Study Design – not clear**
3. **Methodology – it should be clear with rosemary's different doses and number of groups and control for better understanding**
4. **Results –**
5. **Conclusion – its contradicting the findings from results, If the results showed inhibition of p53 expression, the conclusion should reflect that.**

Overall, consistency should be maintained in the manuscript, always use scientific names, such as always referring to *Rosmarinus officinalis* in italics and using the common abbreviation "DMBA" after its full mention.
Break longer sentences to short briefs for the good readability of a reader.

1. Aim : This research aims to test whether the administration of ethanol extract from Rosmarinus officinalis (rosemary) inhibits the expression of Arginase-1 and enhances Tumor Suppressor p53 in Hepatocellular Carcinoma (HCC) induced in male Wistar rats by p-Dimethylamino Benzaldehyde (DMBA).
2. This study used an in vivo experimental design to evaluate the effects of rosemary leaf extract.
3. Wistar rats were induced with p-Dimethylamino Benzaldehyde (DMBA) and treated with varying doses of ethanol extract from rosemary leaves (200 mg/kg BW, 400 mg/kg BW, and 800 mg/kg BW). A control group was included, and the experiment was replicated five times. Hepatocellular carcinoma in liver tissue samples was evaluated through immunohistochemistry for Arginase-1 and Tumor Suppressor p53 expression. Histochemical scoring (H-score) was used to interpret immunoreactivity
4. The results showed that the ethanol extract of rosemary leaves inhibited the decrease in Tumor Suppressor p53 expression in the DMBA-treated group. Significant increases in p53 expression were observed at doses of 400 mg/kg and 800 mg/kg BW. One-way ANOVA of the immunohistochemical tests for Arginase-1 and p53 revealed a significant difference between groups ($p < 0.05$).
The ethanol extract of rosemary leaves demonstrated a potential to inhibit the decrease in Tumor Suppressor p53 expression but did not significantly affect the expression of Arginase-1 in hepatocellular carcinoma in male Wistar rats induced by p-Dimethylamino Benzaldehyde (DMBA) | |
| Is the manuscript scientifically, correct? Please write here. | Its good and correct, but there are a few areas where clarification or further detail could improve its accuracy and overall quality

Areas for Improvement:
1.   Clarity on Results and Conclusion:
  o The conclusion seems somewhat contradictory. The results suggest that the rosemary extract inhibited the decrease in p53 expression, while the conclusion states that the extract could not inhibit the increase in p53 expression. These two statements should be aligned. If the extract shows a positive effect on p53, the conclusion should reflect that, such as stating it "enhanced the expression of p53."
  o The impact on Arginase-1 expression should also be clarified. The conclusion implies that the extract did not significantly affect Arginase-1, but the results do not clearly state whether there was no effect or just a minor one. Be specific about whether the extract had no effect or just an insignificant effect on Arginase-1 expression.
2. Control Groups: The methodology mentions "normal and negative controls," but it is unclear what these groups consist of. It would be beneficial to specify the exact groups used for comparison, such as a negative control group (untreated or saline-treated rats) and a positive control group (rats treated with an established cancer treatment).
3. Dose Rationale: While the study employs varying doses of rosemary leaf extract (200 mg/kg, 400 mg/kg, 800 mg/kg), the rationale behind selecting these particular doses is not mentioned. Are these doses based on previous studies? Providing a brief explanation for dose selection would strengthen the manuscript scientifically.
4. Histochemical Scoring (H-score): The methodology mentions that H-score was used to interpret immunoreactivity, but it would be helpful to briefly describe how the H-score is calculated and its significance in the context of this study. This will ensure that the readers understand the evaluation criteria used to measure p53 and Arginase-1 expression. | |

	5. Study Duration and Sample Size: The study duration (December 2023 to July 2024) and sample size (replication carried out 5 times) are mentioned, but more details on the total number of animals used in each group would be useful for clarity. A larger sample size may be necessary to establish statistical significance. 6. Statistical Analysis: The manuscript mentions using One-way ANOVA to analyze the results, but it would be helpful to add more detail about the statistical analysis, such as how the data were distributed and whether post hoc tests were conducted to determine the specific differences between groups. Recommendations for Improvement: 1. Alignment of Results and Conclusion: Ensure that the results and conclusion are consistent. If rosemary extract showed a positive effect on p53, it should be reflected in the conclusion, and vice versa for Arginase-1. 2. Clarify Control Groups and Sample Sizes: Provide more details on control groups and the number of animals per group to enhance transparency. 3. Provide Rationale for Dose Selection: Briefly mention why the selected doses were used, based on previous studies or literature. 4. Elaborate on Statistical Methods: Add details about the statistical analysis, especially if post hoc tests were performed after ANOVA.	
Are the references sufficient and recent? If you have suggestions of additional references, please mention them in the review form.	The references included are generally appropriate for the topics covered in the manuscript, including the role of rosemary in medicinal applications, hepatocellular carcinoma, antioxidant activity, and specific biomarkers like Arginase-1 and p53. • Some of the references, such as those from 2016-2020, may not fully represent the most current advances in the field, especially in fast-moving areas like cancer treatment, biomarker analysis, and the use of natural compounds in medicine. • Including more recent studies (from 2021-2023) would make the article more up to date. Adding few additional references as **Rosemary as an Anticancer Agent**: • A study examining the potential anticancer effects of rosemary extracts and polyphenols, including carnosic acid and rosmarinic acid, on hepatocellular carcinoma or other cancer models. ○ E.g., *"The anticancer effects of rosemary (Rosmarinus officinalis) and its bioactive compounds in cancer therapy: Current evidence and future prospects."* (2022) **Recent Advances in Cancer Biomarkers**: • New research on cancer biomarkers in liver diseases, particularly in hepatocellular carcinoma. ○ E.g., *"Biomarkers in hepatocellular carcinoma: Advances in diagnosis and prognosis."* (2023) **Immunotherapy and Tumor Suppressor Genes**: • Studies linking the tumor suppressor p53 to cancer immunotherapy, particularly with regard to its role in liver cancer. ○ E.g., *"p53 and cancer immunotherapy: Current developments and future directions."* (2022) **Antioxidants and Their Mechanisms in Cancer Therapy**: • More recent studies focusing on the mechanisms of action of antioxidants in cancer therapy. ○ E.g., *"Mechanisms of antioxidant action in cancer therapy: A focus on herbal extracts and polyphenols."* (2023) **Recent Findings on Arginase-1 in Liver Cancer**: • Studies detailing the role of Arginase-1 in the progression of hepatocellular carcinoma and its potential as a therapeutic target. ○ E.g., *"Targeting Arginase-1 in liver cancer: A new avenue for immunotherapy."* (2023) **Methodology in Herbal Extracts**: • Studies that focus on the extraction methods of bioactive compounds from plants, including rosemary, and their efficacy in medicinal applications. ○ E.g., *"Multilevel maceration for the extraction of polyphenols and flavonoids from medicinal plants."* (2021)	

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Is the language/English quality of the article suitable for scholarly communications?	Its generally well written and clear, but it can overall be refined, readability and precision. Some sentences could be restructured for clarity and to avoid awkward phrasing. Avoid tenses with past and future to maintain consistency Remove unnecessary repetition to ensure that key points stand out. **Example Edits:** **Original:** "The organoleptic properties of rosemary leaf ethanol extract were identified visually. The observation results showed that the rosemary leaf ethanol extract had a blackish brown color. If the extract is open and at room temperature and exposed to light, the extract can absorb oxygen from the air so that it can produce a darker color." **Revised:** "The organoleptic properties of the rosemary leaf ethanol extract were visually assessed. It was observed that the extract had a blackish-brown color. When exposed to air and light at room temperature, the extract absorbed oxygen, resulting in a darker color." **Original:** "The selection of multilevel maceration method in this study was to separate the antioxidant compounds contained in the rosemary leaf sample from other substances according to their polarity level." **Revised:** "In this study, a multilevel maceration method was chosen to separate the antioxidant compounds in the rosemary leaf extract based on their polarity." **Original:** "Albino rats (Rattus norvegicus L.), better known as laboratory rats, are used as a research model in biomedicine." **Revised:** "Albino rats (Rattus norvegicus L.), commonly used as laboratory models in biomedicine, were employed in this study."	
Optional/General comments	• Overall, the manuscript is strong and well-structured. With a few adjustments to improve clarity, the addition of recent references • More of plural words are added in materials and methodology • All ethical approval statement, as this adds credibility to the study. Ensure that the ethical approval details For example object glasses, cover glasses , droppers, etc hardware details are not required **Major Revision is recommended, especially in terms of clarity, more thorough discussion, and further enhancement of the manuscript's structure.** • The research addresses a relevant topic with potential therapeutic implications. • The study design is sound and appropriate for the objectives. • Some key experimental data are presented well (e.g., immunohistochemistry results, statistical analysis). • The clarity and readability of the manuscript could be improved in some sections. • More detailed discussion on the implications of the findings in relation to existing literature could enhance the overall value. • References should be updated to include the most recent studies in the field. The methodology section could benefit from more detail, particularly on the treatment protocol and the reasoning behind dosage selection.	

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	Reviewer's comment	Author's comment *(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Are there ethical issues in this manuscript?	*(If yes, Kindly please write down the ethical issues here in details)*	

Reviewer Details:

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